

## Method of removing N-terminal alanine residues from polypeptides with *Aeromonas* aminopeptidase

### *Abstract*

This invention describes a method of removing N-terminal alanine residues  
5 from polypeptides, preferably recombinant proteins, using an aminopeptidase  
derived from the marine bacterium *Aeromonas proteolytica*. Accordingly,  
*Aeromonas* aminopeptidase (AAP; E.C. 3.4.11.10) can be used to remove N-  
terminal alanyl residues from derivatives of human somatotropin (hST, human  
10 growth hormone, or hGH), porcine somatotropin (pST), and bovine somatotropin  
(bST), for example, to yield proteins having their native amino acid sequences. The  
enzyme reactions can be carried out in free solution, or the AAP can be  
immobilized on a solid support, for reactions carried out *in vitro*. An efficient  
method for converting Ala-hGH to hGH, for example, comprises expression of Ala-  
hGH in *E. coli*, recovery of inclusion bodies, solubilization and refolding in  
15 detergent, detergent removal by ultrafiltration, selective precipitation, enzyme  
cleavage, followed by two column chromatography steps.